

The Influence of Confounding Factors on Algal Response to Nutrients in Lakes: Implications for Nutrient Criteria

Abstract

National Lakes Assessment data from the 2007 and 2012 surveys were used to evaluate the influence of confounding factors on algal response to nutrients. Nitrogen (how it affects chl:TP), phosphorus (how it affects chl:TN), non-algal turbidity, and surface temperature were evaluated. NLA data were used to develop “high yield” chl:TP, chl:TN, and secchi:chl regression lines. Using these benchmark lines to define a high yield of chl-a (at sample TP and TN), the combined influence of all confounding factors was characterized based on the degree to which observed chl-a differs from expectations (chl-a O/E ratio). This approach suggests that chl-a response to nitrogen progressively declines at N:P ratios above approximately 15 (mass units), an indication that phosphorus is typically (or more often) the limiting nutrient. Similarly, chl-a response to phosphorus progressively declines at N:P below approximately 15, an indication that nitrogen is typically (or more often) the limiting nutrient. Multiple linear regression indicates that in addition to N:P ratios, non-algal light attenuation/turbidity and surface temperature also influence algal response to nutrients, and help explain both cross-region and intra-region variation in mean chl:TP and chl:TN yields. Generally, NLA data indicate that when deriving nutrient criteria based on empirical algal response relationships, it is important to consider the potential influence of confounding factors.

Introduction

Resource limitation theory (based on Liebig’s “law of the minimum”) provides that the resource in shortest supply will limit growth. Phosphorus has long been considered an important limiting factor in freshwater lakes (Schindler 1977) and efforts to reduce or control eutrophication typically have focused on decreasing inputs of phosphorus (Schindler 2008). However, experimental evidence also supports the view that nitrogen is an important limiting factor in freshwater lakes, individually or in combination with phosphorus (Elser et al. 1990, Elser et al. 2007, Lewis and Wurtsbaugh 2008, Sterner 2008), and that a dual control strategy is appropriate for protection of freshwater, coastal and marine systems (Paerl 2009, Lewis et al. 2011, U.S. EPA 2015).

Although nutrients are often considered to set an upper limit on lake algal biomass, it is well established that other factors may suppress or limit algal response to nutrients (Bachmann 2001). A partial list of factors that contribute to variation includes physical factors such as non-algal turbidity (Jones et al. 2008a, Dzialowski et al. 2011), lake depth (Philips et al. 2008), mixing status (Mazumder 1994), hydraulic residence time (Soballe and Kimmel 1987, Jones et al. 2008b), temperature (Beaulieu et al. 2013), and latitude (Abell et al. 2012), and biological factors such as zooplankton grazing (Lampert et al. 1986, Mazumder and Havens 1998), self-shading (Wetzel 1966), and the chlorophyll content of algae (Nicholls and Dillon 1978). The influence of such factors helps explain why chlorophyll levels vary substantially at any given TP or TN concentration (Ahlgren et al. 1988) Knowlton and Jones 2006).

Eutrophication studies in the 1960s and 1970s included evaluation of data from northern, temperate, natural lakes where phosphorus levels are low and transparency is typically dominated by phytoplankton. Phosphorus

is often limiting in such lakes, and cross-lake chl:TP relationships are typically linear (log-log) and strongly correlated (Dillon and Rigler 1974, Jones and Bachmann 1976, Carlson 1977).

Reservoirs may exhibit algal responses that differ from those observed in natural lakes (Canfield and Bachmann 1981). Walker (1982) evaluated data from reservoirs located throughout the U.S. and concluded that nitrogen, non-algal turbidity, and temperature have an important influence on the chl-a vs TP relationship. He found that screening the reservoir dataset to eliminate station-years with low inorganic nitrogen/orthophosphate ratios (indicating N-limitation), or high non-algal turbidity levels resulted in a chl-a vs TP model nearly identical to those for northern, temperate, natural lakes (e.g., the line in Jones and Bachmann 1976). Similarly, a cooperative worldwide project to study eutrophication across a diverse set of conditions excluded some lakes from the derivation of chl-a vs TP regression equations based on indications that algae were limited by factors other than phosphorus (OECD 1982). Judgments regarding the likelihood of phosphorus versus nitrogen limitation were based primarily on the ratio of inorganic nitrogen to orthophosphate concentrations (except that the Nordic Project also considered bioassay results).

Total measurements of nitrogen and phosphorus (N:P ratios) have been used as indicators of nutrient limitations (Smith 2006). High ratios (e.g., > 25:1) have been recommended as indicators of “phosphorus-only” limitation, low ratios (e.g., < 10:1) have been recommended as indicators of “nitrogen-only” limitation, and intermediate N:P ratios have been recommended as indicators of co-limitation (Table 1). With respect to deviations from Sakamoto’s chl:TP regression line, Dillon and Rigler (1974) noted that “lakes with an N:P ratio greater than 12 deviated from his line only slightly, while those with low N:P ratios showed larger deviations; the reverse of this was true for a chlorophyll vs nitrogen plot.” Nitrogen-only limitation may be a characteristic of lakes where inflows are dominated by phosphorus-rich discharges from domestic wastewater treatment plants (Downing and McCauley 1992, Lewis and Wurtsbaugh 2008). Conversely, phosphorus-only limitation may be a characteristic of lakes affected by heavy atmospheric deposition of nitrogen (Bergström and Jansson 2006, Elser et al. 2009).

Table 1 N:P Ratios (by Mass) Suggested as Indicators of Nutrient Limitations			
Source	P-Limitation	Co-Limitation (N and P)	N-Limitation
Sakamoto 1966	> 17:1	17:1 to 10:1	< 10:1
Forsberg and Ryding 1980	> 17:1	17:1 to 10:1	< 10:1
Morris and Lewis 1988	> 25:1	25:1 to 15:1	< 15:1
Dzialowski et al. 2005	> 29:1	21:1 to 9:1	< 8:1

Several researchers have demonstrated that when using large compilations of data from lakes with wide-ranging characteristics, a log-log chl:TP plot can have a sigmoidal or nonlinear response pattern (Forsberg and Ryding 1980, McCauley et al. 1989, Jones et al. 2011). Potential causative factors have been evaluated (Chow-Fraser et al. 1994). Stauffer (1991) cautioned that “the sigmoidal chlorophyll *a* v. TP response curve, when fitted indiscriminately using a straight line, can lead to biased predictions concerning lake trophic state.”

Four questions are addressed below:

- (1) does the N:P ratio influence the algal yield, per unit of TP or TN, that can be expected?
- (2) do non-algal light attenuation/turbidity and temperature also affect algal response to nutrients?
- (3) are there ecoregional differences?
- (4) what are the implications for derivation of lake nutrient criteria?

Method

National Lakes Assessment surveys completed in 2007 (U.S. EPA 2009) and 2012 provide 1,985 samples with paired results for chlorophyll-a (chl-a), secchi depth, TP, TN, and surface temperature. A wide range of conditions is represented (chl-a from 0.1 to 940 µg/L, secchi depth from 0.02 to 37 meters, TP from 1 to 4,900 µg/L, TN from 5 to 54,000 µg/L, and surface temperature from 9.6 to 38 °C). For 7% of these samples (n = 142), chl-a, TP, or TN concentrations were less than the reporting limit.

“High yield” regression lines were developed using chl:TP, chl:TN, and secchi:chl-a pairs, excluding samples where chl-a, TP, or TN were below the reporting limit. For each line, the samples were sorted and divided into equal size bins (~200 samples per bin). For the chl-a lines, bin 95th percentile concentrations were calculated, and for the secchi depth line (to improve correlation), bin 90th percentile concentrations were calculated. Regression of the upper percentile values (against bin geometric mean TP, TN, or chl-a) produced the equations below (plotted in Figure 1).

- (1) $(\text{Chl } 95^{\text{th}})_{\log} = 0.9924 \cdot \text{TP}_{\log} - 0.00349$ ($R^2 = 0.99$, 9 bins, 1,846 samples)
- (2) $(\text{Chl } 95^{\text{th}})_{\log} = 1.171 \cdot \text{TN}_{\log} - 1.659$ ($R^2 = 0.99$, 10 bins, 1,926 samples)
- (3) $(\text{Secchi } 90^{\text{th}})_{\log} = -0.5357 \cdot \text{Chl}_{\log} + 0.9338$ ($R^2 = 0.99$, 10 bins, 1,929 samples)

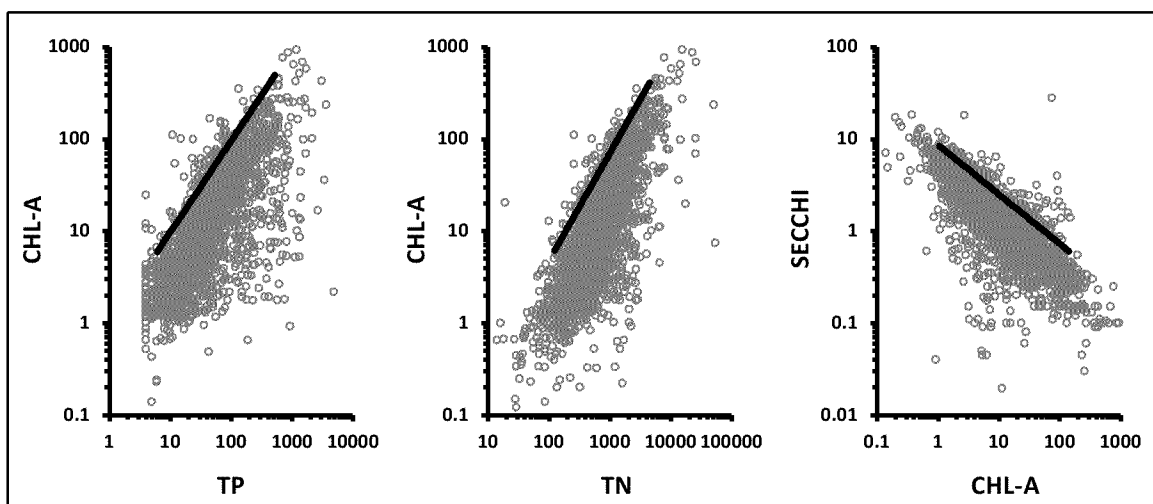


Figure 1. Chl:TP, Chl:TN, and Secchi:Chl “high yield” regression lines. 2007 and 2012 NLA survey data.

Equations 1 and 2 provide estimates of near maximum chl-a levels that can be expected when the nutrient of concern is limiting and conditions are favorable for algal growth (at any given TP or TN concentration). The contrast between the chl-a observed versus that expected based on the benchmark line (chl-a O/E ratio) indicates the cumulative influence of factors that suppress/limit algal growth (confounding factors). Similarly,

non-algal light attenuation/turbidity can be estimated based on the extent to which observed secchi depth deviates from the equation 3 prediction (secchi O/E ratio). The premise of this approach is that deviations are a function of the non-algal component of transparency. These methods are similar to those used in Brown et al. (2000), Jones et al. (2008a), Jones and Hubbart (2011), and also the Carlson trophic state index (TSI) deviation method discussed in Carlson and Havens (2005).

Does the N:P Ratio Influence the Algal Yield, Per Unit of TP or TN, that can be Expected?

Comparison of chlorophyll-a O/E values suggests that N:P ratios (mass units) can be used to predict whether chl:TP response is affected by nitrogen, or chl:TN by phosphorus. Figure 2 (top panels) plots chlorophyll-a O/E values against N:P. Overlaid are bin geometric mean O/E values at bin geometric mean N:P (approximately 200 samples per bin). The comparison shows that O/E (TP) steadily declines across the low N:P range, while O/E (TN) steadily declines across the high N:P range.

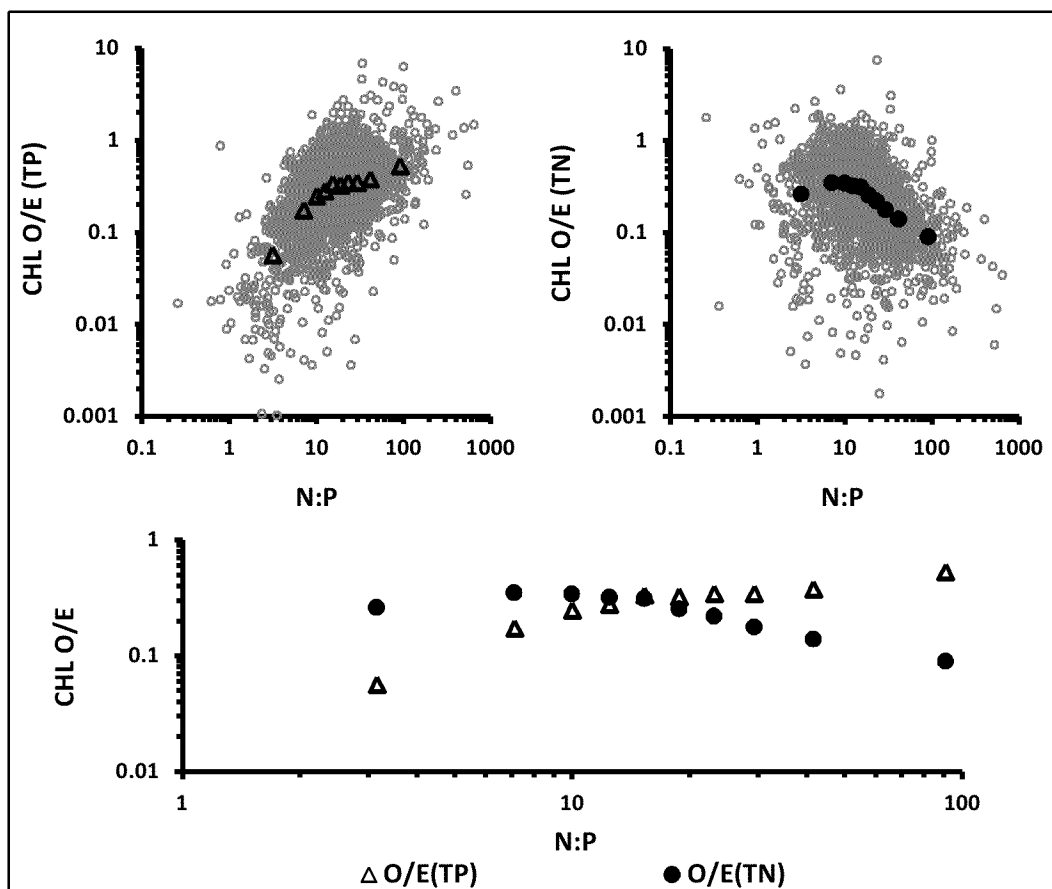


Figure 2. In the top panels, Chlorophyll-a O/E(TP) and O/E(TN) values are plotted against N:P ratio (mass units). Bin geometric means are overlaid. In the bottom panel, both sets of geometric mean O/E values are plotted against bin geometric mean N:P ratio ($n = 1,985$ NLA samples).

The bottom panel compares both sets of geomean chl-a O/E values, and shows that at intermediate ratios (approximately $10 < \text{N:P} < 21$), O/E values are similar using either TP or TN. Parity is reached at an N:P of about 15:1. Below the intermediate range, TN-based O/E values are higher (suggesting TP is present in excess) and above the intermediate range, TP-based O/E values are higher (suggesting TN is present in excess). The mirror

image pattern suggests that N:P ratios provide a useful indicator of the confounding influence of nitrogen (on chl:TP response) and phosphorus (on chl:TN response). That the symmetry is imperfect suggests that non-nutrient factors (light, temperature, etc.) also influence algal response.

Although high and low N:P ratios appear to indicate phosphorus and nitrogen limitation, respectively, it is well established that total measurements are not necessarily good surrogates for the bioavailable phosphorus and nitrogen fractions. Thus using N:P ratios to predict nutrient limitations may be problematic in some cases (Morris and Lewis 1988). In addition, where phosphorus and nitrogen concentrations are both so high as to be above saturation, the utility of the N:P ratio breaks down, and non-nutrient factors such as self-shading (Wetzel 1966) may limit algal biomass. The predictive accuracy of N:P ratios and other indicators (e.g., including those based on dissolved nutrient concentrations) can be evaluated using nutrient enrichment study results. For example, Carlson and Havens (2005) compares N:P ratios to the results from nutrient enrichment studies and concludes that decreasing N:P indicates an increasing likelihood of nitrogen limitation. Similarly, Downing and McCauley (1992) concludes that nitrogen limitation is likely at N:P ratios less than 14:1 (especially where TP > 30 µg/L). These findings are consistent with Figure 2. For example, at N:P ratios less than 15:1, chl-a O/E based on TN exceed those based on TP (on average), and at N:P ratios less than 10:1, nearly all NLA samples (497/498) have O/E (TN) > O/E (TP). Similarly, at N:P ratios greater than 21:1, all samples (785/785) have O/E (TP) > O/E (TN).

Do Non-Algal Light Attenuation and Temperature also Influence Algal Response to Nutrients?

Multiple linear regression of NLA data indicates that in addition to N:P ratios, non-algal light attenuation/turbidity and surface temperature also significantly influence chl:TP and chl:TN response. Table 2 presents the MLR equations using individual observations (n = 1,985). For the analysis, secchi O/E (based on Equation 3) was used as a surrogate indicator of non-algal light attenuation/turbidity.

With individual observations, TP_{log} (R² = 0.54) and TN_{log} (R² = 0.58) perform similarly as predictors of Chl_{log} (equation 4 and 5). However, both models are improved (to R² = 0.70) by adding N:P_{log}, secchi O/E_{log}, and surface temperature. With equation 6 and 7, all four predictor variables are highly significant (p < 0.01). Although not shown, similar regression equations (using the same four highly significant predictor variables) are produced using an adjusted dataset (n = 1,843) that excludes samples with less than reporting limit concentrations for chl-a, TP, or TN (n = 142).

Using equation 6 or 7, if TP (geomean = 38 µg/L), TN (geomean = 632 µg/L), N:P ratio (geomean = 17:1), and surface temperature (mean = 24.3 °C) are assumed to equal typical values, and secchi O/E is assumed to increase from the 10th percentile (0.24) to the 90th percentile (0.98), predicted chl-a doubles (from 7 to 14 µg/L). Similarly, if typical values are assumed for all variables including secchi O/E (geomean = 0.51), but surface temperature is assumed to increase from the 10th percentile (18.6 °C) to the 90th percentile (29.6 °C), predicted chl-a again approximately doubles (from 7 to 13 µg/L).

Dividing all samples into three subsets with low, intermediate, and high N:P indicates that secchi O/E and surface temperature have an important influence on chlorophyll-a with each subset (i.e., regardless of which nutrient is indicated as the predominant limiting factor).

- **$N:P < 10$ ($n = 498$).** TN_{log} , secchi O/E_{log} , and surface temperature are highly significant predictors of Chl_{log} , but TP_{log} is not significant when added to the model.
- **$10 \leq N:P < 21$ ($n = 700$).** TP_{log} or TN_{log} , along with secchi O/E_{log} and surface temperature, are highly significant. TP_{log} and TN_{log} are well-correlated and either can be used to predict Chl_{log} .
- **$N:P \geq 21$ ($n = 787$).** TP_{log} is a better predictor than TN_{log} , but TN_{log} remains highly significant when added to the model, as does secchi O/E_{log} and surface temperature.

Generally, the MLR analysis using individual observations suggests that chl-a increases with rising N:P using TP as a predictor, chl-a increases with falling N:P using TN as a predictor, and non-algal light attenuation and surface temperature also have a highly significant influence on the algal biomass that can be expected in lakes.

Table 2 Regression Models of Variation in Chlorophyll Considering TP or TN, N:P, Secchi O/E, and Surface Temperature		
Individual Observations ($n = 1,985$)	Adj. R^2	RMSE
(4) $Chl_{log} = 0.7672*TP_{log} - 0.2241$	0.54	0.44
(5) $Chl_{log} = 1.080*TN_{log} - 2.040$	0.58	0.43
(6) $Chl_{log} = 1.10*TP_{log} + 0.6042*N:P_{log} + 0.5255*Secchi\ O/E_{log} + 0.0268*Surface\ T - 1.987$	0.70	0.36
(7) $Chl_{log} = 1.10*TN_{log} - 0.4955*N:P_{log} + 0.5255*Secchi\ O/E_{log} + 0.0268*Surface\ T - 1.987$	0.70	0.36
Un-Aggregated Omernik Level III Ecoregion Means ($n = 85$)	Adj. R^2	RMSE
(8) $Chl_{log} = 0.8924*TP_{log} - 0.4298$	0.62	0.29
(9) $Chl_{log} = 1.224*TN_{log} - 2.398$	0.75	0.23
(10) $Chl_{log} = 1.172*TP_{log} + 0.6490*N:P_{log} + 0.5767*Secchi\ O/E_{log} + 0.0427*Surface\ T - 2.501$	0.86	0.17
(11) $Chl_{log} = 1.172*TN_{log} - 0.5232*N:P_{log} + 0.5767*Secchi\ O/E_{log} + 0.0427*Surface\ T - 2.501$	0.86	0.17
Un-Aggregated Omernik Level III Ecoregion Means (Min 5 samples, $n = 68$)	Adj. R^2	RMSE
(12) $Chl_{log} = 0.9022*TP_{log} - 0.4685$	0.67	0.25
(13) $Chl_{log} = 1.179*TN_{log} - 2.272$	0.80	0.20
(14) $Chl_{log} = 1.091*TP_{log} + 0.5403*N:P_{log} + 0.6129*Secchi\ O/E_{log} + 0.0483*Surface\ T - 2.380$	0.92	0.12
(15) $Chl_{log} = 1.091*TN_{log} - 0.5509*N:P_{log} + 0.6129*Secchi\ O/E_{log} + 0.0483*Surface\ T - 2.380$	0.92	0.12

Are There Ecoregional Differences?

Box and whisker plots (Figure 3) for the 9 aggregated Omernik Level III ecoregions (mapped in Figure 20, U.S. EPA 2009) illustrate cross-region differences in N:P ratios, secchi O/E values, and surface temperatures.

- N:P ratios are elevated in the Upper Midwest and Northern Appalachians (i.e., the median N:P ratio is 28:1, with 67% of N:P ratios $> 21:1$). However, nutrient levels are more balanced in the remainder of the U.S. (median N:P = 14:1, 30% of samples have an N:P ratio > 21 , 32% of samples have an N:P ratio < 10). Put another way, 35 $\mu\text{g/L}$ TP (the median of all samples) relates to an N:P ratio of 25:1 in the Upper Midwest, 17:1 in the Coastal Plains, and 10:1 in the Western Mountains.
- Non-algal light attenuation/turbidity also varies regionally (indicated by secchi O/E). Non-algal turbidity is typically low in the Western Mountains (median secchi O/E = 0.69) but commonly elevated in the Southern Plains (median = 0.35). Put another way, 10 $\mu\text{g/L}$ chl-a relates to 1.7 meters of secchi depth in the Western Mountains, on average, but just 0.72 meters in the Southern Plains.
- Surface temperatures are typically cold in the Western Mountains (median = 19.8 °C) and warm in the Coastal Plains (median = 29.2 °C).

- Chl-a O/E values (Table 3) indicate that algal responses also vary across the ecoregions, with the strongest responses observed in the Coastal Plains (both nutrients). For example, 35 µg/L TP relates to 16 µg/L chl-a in the Coastal Plains but 5 µg/L chl-a in the Western Mountains, and 600 µg/L TN relates to 17 µg/L chl-a in the Coastal Plains but 5 µg/L chl-a in the Northern Plains. Note that algal response (both nutrients) is suppressed in ecoregions with cold surface temperatures, regardless of whether nutrient concentrations are low (Western Mountains) or high (Northern Plains).

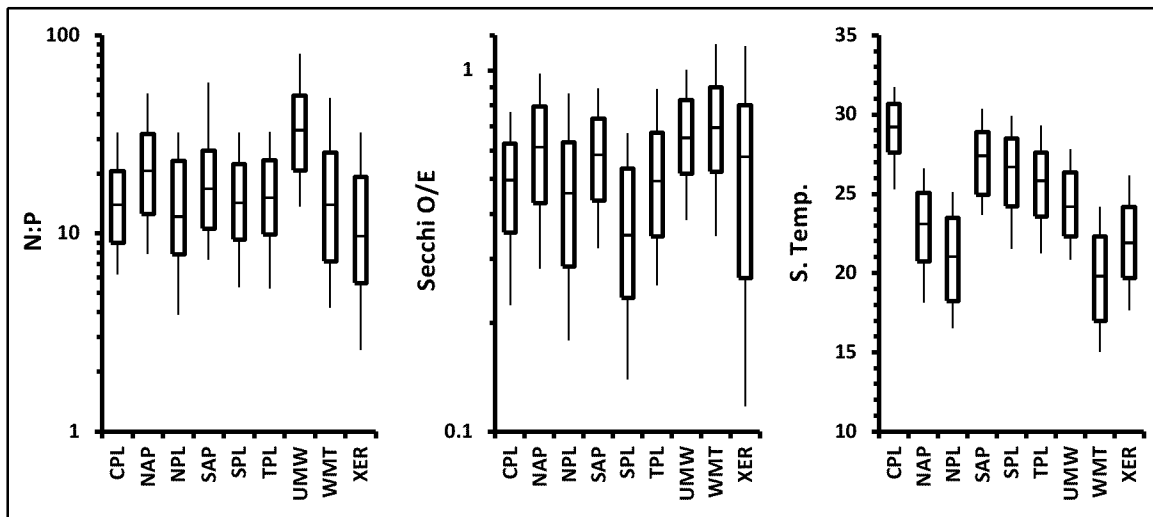


Figure 3. Box and whisker plots for the aggregated Omernik Level III ecoregions. Boxes represent the 25th, 50th, and 75th percentiles, and whiskers represent the 90th and 10th percentiles. Ecoregion abbreviations are spelled out in Table 3.

Ecoregion	N	O/E (TP)	O/E (TN)	Chl-a	TP	TN	SD O/E	Surface T	N:P	% > 21	% < 10
Coastal Plains	236	0.43	0.47	25	54	800	0.50	29.2	13.9	25%	31%
Northern Appalachians	209	0.34	0.23	3.6	13	300	0.68	23.8	24.1	58%	6%
Northern Plains	106	0.14	0.13	15	135	2000	0.46	21.0	12.2	30%	37%
Southern Appalachians	207	0.41	0.37	6.5	19	370	0.58	27.4	16.8	37%	22%
Southern Plains	203	0.29	0.25	19	88	950	0.35	26.7	14.3	29%	29%
Temperate Plains	291	0.37	0.33	27	89	1300	0.49	25.8	15.2	33%	25%
Upper Midwest	285	0.38	0.15	5.8	20	640	0.65	24.2	33.2	74%	3%
Western Mountains	281	0.18	0.22	2.4	20	250	0.69	19.8	13.9	33%	36%
Xeric West	163	0.18	0.21	6.1	53	560	0.58	21.9	9.7	23%	52%
Hawaii	4	0.62	0.77	31	48	550	0.59	24.7	12.3	0%	50%
Grand Totals	1985	0.32	0.25	8.6	35	600	0.57	24.6	16.9	40%	25%

Table 2 identifies multiple linear regression equations using mean values for the individual Level III ecoregions ($n = 85$, an average of 23 observations per ecoregion). With ecoregion means, Chl_{\log} can be predicted using only TP_{\log} (adjusted $R^2 = 0.62$, equation 8) or only TN_{\log} (adjusted $R^2 = 0.75$, equation 9). The chl:TP and chl:TN models are improved (to $R^2 = 0.77$ and 0.81 , respectively) by adding surface temperature, but further improvement is achieved (to $R^2 = 0.86$ for both) by also adding secchi O/E_{\log} and N:P_{\log} terms (equations 10 and 11, all four predictor variables are highly significant, $p < 0.01$). Excluding ecoregion means based on fewer than five samples improves correlation and reduces standard error (equations 12 – 15, $n = 68$, an average of 29

observations per ecoregion). This approach excludes data poor ecoregions ($n < 5$), including the “Hawaii” ecoregion.

Generally, the MLR analysis using ecoregion means shows that chl-a predictions can be significantly improved by accounting for cross-region differences in N:P ratio, non-algal turbidity, and surface temperature. However, other factors (e.g., zooplankton grazing, algal speciation, hydraulic residence time, depth, mixing, etc.) were not evaluated and might also be important.

What are the Implications for Derivation of Lake Nutrient Criteria?

National Lakes Assessment data indicate that algal responses to nutrients vary across the ecoregions, due to the influence of multiple factors that also vary across the ecoregions. This has several important implications for derivation of nutrient criteria.

One-Size-Fits-All Models

For purposes of translating from chlorophyll-a to nutrient targets, a one-size-fits-all chl:TP or chl:TN model may be problematic, particularly with datasets spanning a wide range of nutrient concentrations and physical conditions. Consider that with equations 4 and 5 (Table 2), 10 $\mu\text{g/L}$ chl-a (Walker 1985) translates to 39 $\mu\text{g/L}$ TP and 650 $\mu\text{g/L}$ TN. Although this may be a reasonable way to estimate the mean chl-a concentrations that can be expected across many lakes, responses in individual lakes will vary substantially. Yields will be higher where conditions are favorable for growth, and lower where confounding factors suppress growth. The implication is that datasets from multiple regions - with different chemical and physical conditions - should not necessarily be combined when developing algal response models, particularly when using only TP or TN as predictor variables.

Lake Classification

Given the influence of confounding factors on algal response to nutrients, a strong case can be made that lake classification should be explored prior to attempting derivation of nutrient criteria using empirical algal response relationships. Classification allows for groupings of lakes with similar chemical and/or physical conditions, so that algal response to nutrients can be predicted with greater confidence and accuracy. This is important if the goal is to derive nutrient criteria that are neither over-protective nor under-protective.

Intra-Region Variability

It is important to consider that confounding factors are a cause of both cross-region *and* intra-region variability. Figure 4 plots chl:TP and chl:TN regression lines for 22 Level III ecoregions, and again shows that there are regional differences. What Figure 4 does not show is that for each of the datasets, the chl:TP and/or chl:TN model is significantly improved by including one or more additional predictor variables. N:P is a statistically significant ($p < 0.05$) additional predictor in 21 of the ecoregions, secchi O/E in 12 ecoregions, and surface temperature in 1 (Northern Rockies). This raises a question about what nutrient criteria derivation methods are appropriate for individual ecoregions where a cross-lake gradient of conditions influences the chl-a levels that can be expected. For example, there is a risk that N-limited conditions or high levels of non-algal turbidity in some lakes may bias chl:TP response predictions for lakes where these confounding influences are not present. Of course, there are other characteristics that may also need to be considered, including temperature (Beaulieu et al. 2013), hydraulic residence time (Jones et al. 2008b), and others.

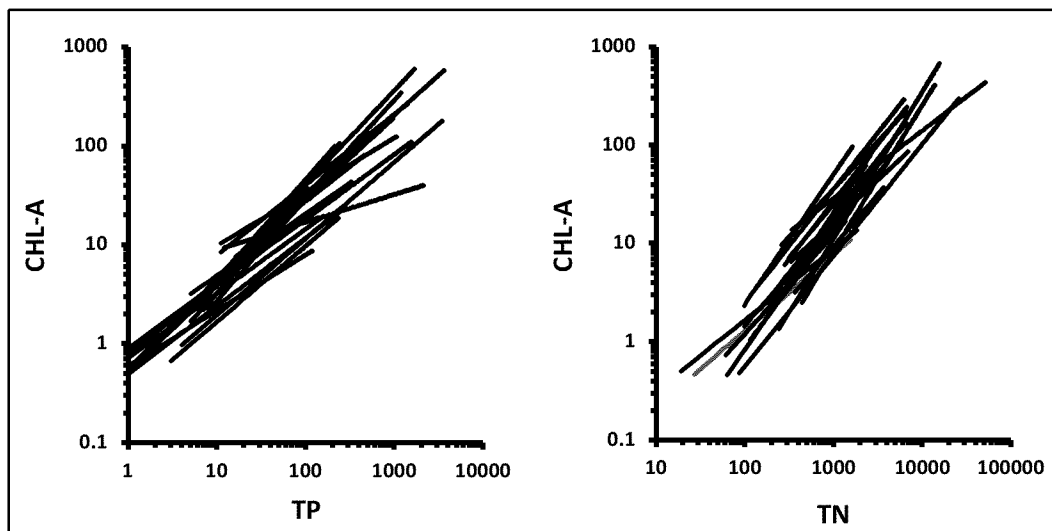


Figure 4. Chl-a vs TP (left) and Chl-a vs TN (right) for 22 Level III ecoregions (minimum of 30 samples with paired chl-a, secchi, TP, TN, and surface temperature).

Time Variability

The influence of a particular confounding factor may vary over the course of a growing season, and the seasonal or long-term average for the response variable may align with expectations, despite strong confounding effects over short time-frames. For example, nitrogen only limitation may be observed in late summer (Morris and Lewis 1988) or throughout the growing season (Lewis et al. 2008). Similarly, the confounding effect of non-algal turbidity may be seasonal/intermittent (Jones and Knowlton 2005) or persistent (Jones et al. 1998, e.g., see the plot for Higginsville Lake). Accordingly, seasonal or longer duration averages should be used for purposes of nutrient criteria, and when making lake classification decisions.

N:P Ratios

N:P was a significant additional predictor of chl-a (with TP or TN) for 21 of the 22 Level III ecoregion datasets (Figure 4), and for each of the aggregated ecoregions. The implication is that it is important to carefully evaluate the intra-region influence of N:P ratios (or similar metrics based on dissolved nutrient concentrations) when deriving TP and TN criteria with empirical models. Lakes where N:P ratios are typically low (< 10:1) are likely to have weak chl:TP response (e.g., algae are limited primarily by nitrogen), and lakes where N:P ratios are typically high (> 21:1) are likely to have weak chl:TN response (e.g., algae are limited primarily by phosphorus). Ignoring these effects may result in biased criteria.

Non-Algal Turbidity

Secchi O/E was a significant additional predictor of chl-a (with TP or TN) for about half of the Level III ecoregion datasets (Figure 4), and for eight of the nine aggregated ecoregions (the exception was the Upper Midwest). This suggests that non-algal turbidity levels may or may not have an important influence on variation in algal biomass within a given ecoregion. Where non-algal turbidity is important, direct measurement of non-algal seston levels (Jones et al. 2008a) may be worthwhile as part of routine lake monitoring programs and/or future national surveys (i.e., so that levels need not be inferred from secchi depth and chl-a, as was done for this study). Consideration should also be given to alternative methods for estimating non-algal turbidity (see Jones and Hubbart 2011).

Surface Temperature

Surface temperature was not typically a significant predictor with the Level III ecoregion datasets (the Northern Rockies was the only exception) or the aggregated Level III ecoregions (the Temperate Plains was the only exception). However, using mean values for the nine aggregated ecoregions, adding a surface temperature term improves the adjusted R^2 of a Chl_{\log} vs TP_{\log} model from 0.64 to 0.96. The implication is that whereas temperature may not have a significant influence within a given ecoregion, over broad spatial scales it appears to be an important driver of variation in the strength of algal response to nutrients.

Chl:TP Response May be Non-Linear

The moving average chl-a vs TP trendline has a sigmoid shape using NLA data (see Figure 5, left panel, grey line). Similar non-linear chl:TP responses have been demonstrated with large regional datasets using both summer averages (Forsberg and Ryding 1980, $n = 4,500$ samples) and summer maxima (Jones et al. 2011, $n = 8,800$ samples). With the NLA dataset, geometric mean chl-a levels are flattened by strong confounding influences across the high TP range. For example, when the trendline is restricted to only those samples where heavy non-algal turbidity (secchi O/E < 0.25) and/or strong N-limitation ($\text{N:P} < 7$) are indicated (lower black line, $n = 415$, 21% of samples), mean chl-a response is reduced (by $\sim 2/3$) across the intermediate TP range, before plateauing at high TP. The change in slope at high TP corresponds to a gradient of increasingly heavy non-algal turbidity (indicated) and strengthening nitrogen-limitation (indicated), with both trends working in combination to de-couple the chl-a vs TP relationship. By contrast, the right panel of Figure 5 shows that the moving average chl-a vs TN trendline stays within a linear ribbon, and excluding samples where heavy non-algal turbidity and/or strong P-limitation ($\text{N:P} > 30$) are indicated ($n = 659$, 33% of samples) has a less dramatic effect (i.e., slope pivots modestly upward).

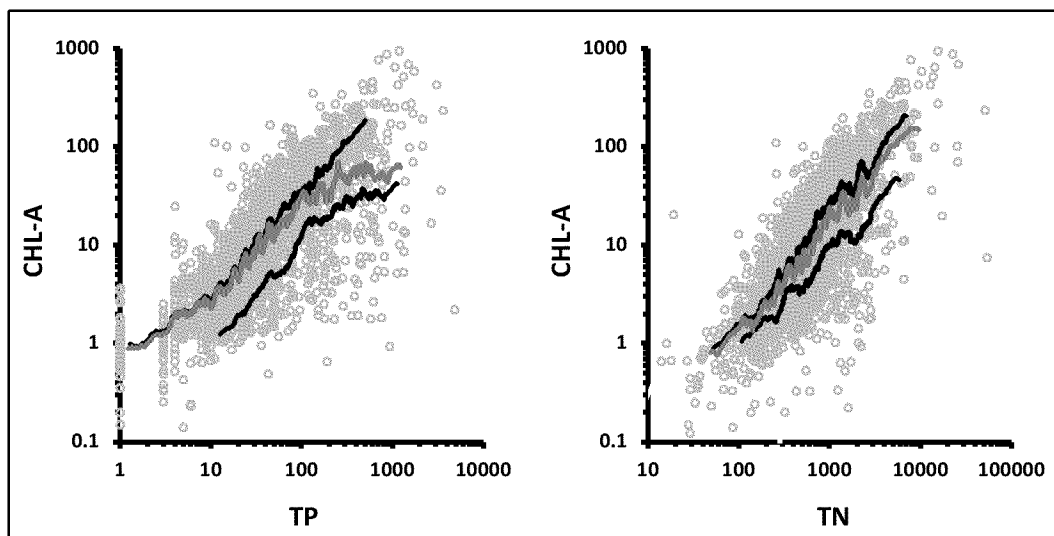


Figure 5. Chl-a vs TP (left) and Chl-a vs TN (right) with three different (50 sample) moving geometric mean trendlines: (1) all data (grey line, $n = 1,985$), (2) only samples where heavy non-algal turbidity (secchi O/E < 0.25) and/or strong limitation by the other nutrient is indicated (lower black line), and (3) samples not meeting either of the screening criteria (upper black line).

The implication is that chl:TP slope may be unstable in regions of the U.S. where nitrogen limitation and/or elevated non-algal turbidity levels are commonly indicated at high TP. In such cases, it may be problematic to

use a linear (log-log) regression model for purposes of describing the response relationship. With regard to calculation of nutrient criteria, the risk is that strong confounding influences at high TP may bias a linear model such that it under-predicts mean chl-a (and chl:TP response ratios) across the intermediate TP range. Removing lakes where strong confounding influences are indicated may be necessary to avoid bias, and would better ensure that TP and TN criteria are protective of the mean chl-a objective. Lakes where strong confounding influences are uncorrectable may need site-specific TP and/or TN criteria.

Conclusion

NLA data highlight some of the challenges that arise when predicting mean algal response to nutrients using a cross-lake data compilation. In particular, it is important to understand that at a given TP or TN concentration, there are likely to be multiple site-specific factors that influence the algal biomass that can be expected (even within a single Level III ecoregion). For purposes of translating chlorophyll-a goals to corresponding nutrient targets, a univariate chl:TP or chl:TN empirical regression approach may be problematic, particularly with datasets spanning a wide range of nutrient concentrations and physical conditions. Ignoring the influence of confounding factors may result in nutrient criteria that do not adequately protect uses, e.g., where the nutrient of concern is limiting or co-limiting and conditions are favorable for algal growth.

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